

## FACTORS AFFECTING SPERMATOZOA MORPHOLOGY IN BEEF BULLS<sup>1</sup>

C. A. Roberts, T. W. Geary, M. D. MacNeil, R. C. Waterman, A. J. Roberts, and L. J. Alexander  
USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, Montana

**ABSTRACT:** The objective of this study was to evaluate factors affecting sperm morphology of bulls (n=908) collected at 320 days of age. Bulls were a composite breed (50% Red Angus, 25% Charolais, and 25% Tarentaise) born from 2002 to 2008 to dams fed levels of feed during mid and late gestation that were expected to provide marginal or adequate nutrition while grazing dormant winter forage. After weaning, bulls were fed to appetite (CON) or restricted (REST) to 80% of that consumed by CON on BW basis. Semen samples were collected using an electroejaculator and evaluated using standard BSE procedures. Spermatozoa morphology was evaluated by classifying 100 spermatozoa per bull at 400 X magnification into the following categories: normal spermatozoa, knobbed acrosome, head defects, distal midpiece reflex, dag defect, bowed midpiece, proximal droplet, distal droplet, coiled principle piece, and bent principle piece. Each morphological trait, along with scrotal circumference (SC), gross motility, and percent progressive motility was analyzed using MTDFREML and pedigree information from 8163 relatives born from 1974 to 2008 to provide heritability estimates. Heritability estimates for these traits were: SC ( $h^2 = 0.67$ ), normal sperm ( $h^2 = 0.18$ ), dag defect ( $h^2 = 0.50$ ), bowed midpiece ( $h^2 = 0.19$ ), proximal droplets ( $h^2 = 0.37$ ), bent principle piece ( $h^2 = 0.18$ ), gross motility ( $h^2 = 0.20$ ), and progressive motility ( $h^2 = 0.20$ ). The moderate heritability of percent normal sperm and several of the other sperm defects suggest that selection for improved sperm morphology is possible. Further analysis with MTDFREML determined genetic correlations between the above traits and pre-weaning gain direct, pre-weaning gain maternal, post-weaning gain, and scrotal circumference. Maternal pre-weaning gain was highly correlated with scrotal circumference ( $r = 0.70 \pm 0.24$ ) but pre-weaning gain direct ( $r = 0.29 \pm 0.20$ ) and post-weaning gain ( $r = 0.01 \pm 0.17$ ) were not. Scrotal circumference and post-weaning gain were not highly correlated with morphology and therefore are not good indicators of spermatozoa morphology. Neither in utero nor postweaning diet affected any of the traits measured.

Key Words: bull, spermatozoa morphology, heritability

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## Introduction

Bull fertility is critical to the economic success of beef cattle operations. Spermatozoa morphology has been shown to be a fundamental element affecting spermatozoa's ability to reach the site of fertilization in the female tract, fertilize an ovum, and initiate embryogenesis (Saacke, 2008). Reproductive traits and seminal quality have not been traditionally subjected to intense selection pressure (Coulter, 1994). The ability to select and breed for a higher percentage of morphologically normal spermatozoa would improve the efficiency and cost effectiveness of beef operations by allowing producers to develop only those bulls with greater potential as successful breeders. The objective of this study was to examine the heritability of traits traditionally associated with bull fertility: scrotal circumference, gross motility, and percent progressive motility; as well as the following morphological characteristics: normal spermatozoa, knobbed acrosome, head defects, distal midpiece reflex, dag defect, bowed midpiece, proximal droplet, distal droplet, coiled principle piece, and bent principle piece. A further objective of this study was to examine the correlations, both phenotypic and genetic, between the following factors and spermatozoa morphology: in utero (dam) nutritional treatment, scrotal circumference, pre-weaning gain direct, pre-weaning gain maternal, and post weaning gain. Establishment of genetic and environmental correlations between traditional measures of bull fertility and spermatozoa morphology could improve interpretation of standard breeding soundness examinations and selection for improved bull fertility.

## Materials and Methods

*Animals, facilities, and diet.* Procedures were approved by USDA-ARS Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. Bulls included in the study (n=908) were a stabilized composite including 50% Red Angus, 25% Charolais, and 25% Tarentaise germplasm. Bulls were evaluated at 320  $\pm$  days of age during a six year period from 2003 to 2009. Bulls were born to dams that were fed levels of harvested feed during mid and late gestation (Dec to March) that were expected to provide marginal (MARG) or adequate (ADEQ) nutrition while grazing dormant winter forage through this period. After weaning, bulls were fed to appetite (CON) or restricted (REST) to 80 % of that consumed by CON on common BW basis until time of evaluation.

*Collection and Sample Evaluation.* Semen was collected from 908 bulls using an electroejaculator. Volume of the ejaculate was recorded. Gross motility (swirl) was evaluated on undiluted semen samples using a light microscope at 100 X magnification. Swirl was evaluated on a 0-5 scale with 0 indicating no movement present and 5 indicating many swirls with rapid and vigorous speed. Percent progressive motility was evaluated in semen samples diluted 1:4 with pre-warmed (37°) Dulbecco's Phosphate-Buffer Saline using 400 X magnification. Percent progressive motility was subjectively scored from 0% to 100% based upon a visual measurement of spermatozoa that exhibited straight-line movement. Concentration of spermatozoa was determined by hemocytometer from neat semen diluted 1:40 in eosin counting solution. Spermatozoa morphology was evaluated from a fixed slide prepared at time of collection using morphology stain. One hundred spermatozoa per bull were evaluated at 400 X magnification for the following morphological characteristics: normal, knobbed acrosome, head defects, distal midpiece reflex, dag defect, bowed midpiece, proximal droplet, distal droplet, coiled principle piece, and bent principle piece. Scrotal circumference was also recorded at time of collection using a scrotal tape.

*Statistics.* Data were initially analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). The model included fixed effects of year, dam treatment (either ADEQ or MARG) and postweaning treatment (either CON or REST) of the bulls, age-of-dam (2, 3, 4,  $\geq 5$  yr), and age of bull in days. Dependent variables were percentage of each morphological characteristic: normal, knobbed acrosome, head defects, distal midpiece reflex, dag defect, bowed midpiece, proximal droplet, distal droplet, coiled principle piece, and bent principle piece, gross motility (swirl), percent progressive motility, scrotal circumference, pre-weaning gain and post-weaning gain.

Subsequently, multiple-trait-derivative-free REML (Boldman et al. 1995) was used to estimate additive genetic and residual variance components in univariate analyses for each sperm morphology trait. The relationship matrix used was derived from 8,163 animals born between 1974 and 2008. Contemporary groups were defined by year and postweaning treatment. Fixed effects were contemporary group, dam treatment and age (linear). In addition to the univariate analysis of sperm morphology traits, additional bivariate analyses were conducted to estimate correlations of the sperm morphology traits with pre-and post-weaning gain and scrotal circumference.

## Results and Discussion

In utero (dam) nutritional treatment (MARG or ADEQ) had no effect on traditional measures of bull fertility or spermatozoa morphology. Post weaning (REST or CON) nutritional treatment affected postweaning growth, scrotal circumference, and had significant, but biologically unimportant, effects on the frequencies of distal midpiece reflex, proximal droplets, and bent principle piece defects. Our data differs from that of Coulter et al., (1997) in that bulls in the current study were fed diets that were on full feed or 80% of full feed. Thus, our CON diet was more

similar to their moderate-energy diet. New data presented here demonstrates that nutrient limitation did not have detrimental effects either.

Heritability estimates for the traits measured are shown in Table 1. The heritability estimate for scrotal circumference is lower than that of Bourdon et al. (1986). The heritability estimate for percent normal spermatozoa was low, but suggests progress could be made in selection for this trait. The major classification for spermatozoa morphology was percent normal. Heritability estimates for percent dag defect and proximal droplets were high, thus producers could apply selection pressure against these traits that, according to Barth and Oko (1989), are known to negatively affect fertility. Considerable debate has existed between the origin of primary versus secondary abnormalities and major and minor spermatozoa defects contributing to fertility and which traits were genetic abnormalities. The heritability estimates of dag defect, bowed midpiece, proximal droplet, and bent principle piece suggest these to be primarily of genetic origin and that bulls with these abnormalities might be expected to not recover from them. Table 1 also identifies percent bowed midpiece and percent bent principle piece as being lowly heritable, but the incidence of these traits is negligible.

Correlations between pre-weaning gain and each of the fertility and spermatozoa traits measured are listed in Table 2. Maternal pre-weaning gain was highly correlated with scrotal circumference ( $r = 0.70 \pm 0.24$ ). Thus selection for increased milk production would be expected to increase scrotal size and selection for increased scrotal size would indirectly increase milk production in daughters. Bourdon and Brinks (1986) reported a similar direct genetic correlation between scrotal circumference and weaning weight ( $r = 0.20 \pm 0.18$ ) as observed in the present study for pre-weaning gain ( $r = 0.29 \pm 0.20$ ). Post-weaning feed levels and gain had no effect on scrotal circumference in the current data but Bourdon and Brinks (1986) demonstrated increased scrotal circumference for bulls receiving full feed compared to either limited or intermediate feed during the post-weaning period. While the genetic correlation between direct pre-weaning gain and bent principle pieces was high, the incidence of bent principle pieces was negligible. Also, Table 3 suggests that selection for increased post-weaning gain would decrease the percentage of bowed midpiece, but the incidence of this trait was also very small.

## Implications

Scrotal circumference and post-weaning gain were not highly correlated with morphology and therefore are not good indicators of spermatozoa morphology. Neither in utero nor postweaning diet affected any of the traits measured. However, the heritability of percent normal sperm and several of the other sperm defects suggest that selection for improved sperm morphology is possible.

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Table 1. Summary of univariate analysis results

Trait	CON $\mu$	RES $\mu$	$\mu$ of CON and RES	Phenotypic variation	Standard Deviation	$h^2$	$h^2$ standard error	Additive Genetic Variance	Residual Variance
Scrotal circumference, cm	30.78	29.98	30.83	5.89	2.43	0.67	0.09	3.95	1.95
% normal	43.54	41.81	42.68	192.67	13.88	0.18	0.07	34.68	157.99
% knobbed acrosome	0.43	0.41	0.42	0.72	0.85	0.02	0.04	0.01	0.71
% head defects	1.65	1.73	1.68	2.75	1.66	0.00	0.05	0.00	2.75
% distal midpiece reflex	2.47	1.98	2.22	10.86	3.30	0.01	0.04	0.11	10.75
% dag defect	6.85	6.70	6.78	46.92	6.85	0.50	0.10	23.46	23.46
% bowed midpiece	14.96	15.90	15.43	127.37	11.29	0.19	0.07	24.20	103.17
% proximal droplet	23.12	20.13	21.62	306.29	17.50	0.37	0.08	113.33	192.96
% distal droplet	3.07	2.92	3.00	12.68	3.56	0.09	0.06	1.14	11.54
% coiled principle piece	0.88	0.77	0.83	2.31	1.52	0.07	0.05	0.16	2.15
% bent principle piece	0.81	0.49	0.65	2.54	1.59	0.18	0.08	0.46	2.08
Gross motility score	1.58	1.58	1.58	0.65	0.81	0.20	0.07	0.12	0.52
% progressive motility	25.21	24.39	24.80	201.35	14.19	0.20	0.08	40.27	161.08

Table 2. Estimates of correlation between components of pre-weaning gain and common measures of bull fertility

Trait	Direct genetic correlation to pre-weaning gain	Maternal genetic correlation to pre-weaning gain	Residual correlation to pre-weaning gain
Scrotal circumference, cm	0.29 ± 0.20	0.70 ± 0.24	0.41 ± 0.13
% normal	0.09 ± 0.29	0.24 ± 0.35	0.03 ± 0.08
% knobbed acrosome	-0.94 ± 1.04	0.37 ± 0.97	0.05 ± 0.07
% distal midpiece reflex	0.64 ± 1.44	-0.80 ± 1.87	-0.04 ± 0.07
% dag defect	0.05 ± 0.25	0.03 ± 0.27	0.03 ± 0.12
% bowed midpiece	0.06 ± 0.28	-0.12 ± 0.35	0 ± 0.08
% proximal droplet	-0.14 ± 0.23	-0.09 ± 0.29	0 ± 0.09
% distal droplet	-0.15 ± 0.35	-0.08 ± 0.46	-0.04 ± 0.07
% coiled principle piece	0.05 ± 0.39	-0.46 ± 0.52	0.01 ± 0.07
% bent principle piece	0.64 ± 0.26	-0.37 ± 0.39	-0.28 ± 0.09
Gross Motility score	-0.02 ± 0.29	0.20 ± 0.38	0.08 ± 0.09
% progressive motility	-0.29 ± 0.29	0.06 ± 0.38	0.09 ± 0.09

Table 3. Estimates of correlation between common measures of bull fertility and post-weaning gain and scrotal circumference

Trait	Genetic correlation to post-weaning gain	Residual correlation to post-weaning gain	Genetic correlation to scrotal circumference	Residual correlation to scrotal circumference
Scrotal circumference, cm	0.01 ± 0.17	0.35 ± 0.10	n/a	n/a
% normal	-0.24 ± 0.24	0.05 ± 0.06	0.06 ± 0.20	0.19 ± 0.10
% knobbed acrosome	0.1 ± 0.67	-0.05 ± 0.06	-0.18 ± 0.54	0.04 ± 0.09
% distal midpiece reflex	0.64 ± 1.44	-0.01 ± 0.05	1.00 ± 2.01	-0.13 ± 0.09
% dag defect	0.07 ± 0.19	-0.03 ± 0.09	0.02 ± 0.20	-0.01 ± 0.02
% bowed midpiece	-0.47 ± 0.22	0.06 ± 0.07	0.04 ± 0.20	0.09 ± 0.10
% proximal droplet	0.30 ± 0.19	-0.07 ± 0.08	-0.16 ± 0.15	-0.10 ± 0.12
% distal droplet	-0.18 ± 0.31	-0.02 ± 0.06	-0.02 ± 0.26	-0.03 ± 0.09
% coiled principle piece	-0.20 ± 0.34	0.02 ± 0.06	-0.40 ± 0.27	0.08 ± 0.09
% bent principle piece	-0.03 ± 0.28	0.01 ± 0.07	0.11 ± 0.22	-0.02 ± 0.11
Gross Motility score	-0.14 ± 0.26	0.01 ± 0.07	0.32 ± 1.36	0.17 ± 0.68
% progressive motility	-0.49 ± 0.25	0.05 ± 0.07	-0.14 ± 0.20	0.21 ± 0.12